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Aspergillus ochraceus 11 alpha-hydroxylase and oxidoreductase

Abstract

The present invention relates to a novel cytochrome P450-like enzyme (Aspergillus ochraceus 11 alpha hydroxylase) and an oxidoreductase (Aspergillus ochraceus oxidoreductase) isolated from cDNA library generated from the mRNA of Aspergillus ochraceus spores. When the cDNA encoding the 11 alpha hydroxylase was co-expressed in Spodoptera frugiperda (Sf-9) insect cells with the cDNA encoding human oxidoreductase as an electron donor, it successfully catalyzed the conversion of the steroid substrate 4-androstene-3,17-dione (AD) to 11 alphahydroxy-AD as determined by HPLC analysis. The invention also relates to nucleic acid molecules associated with or derived from these cDNAs including complements, homologues and fragments thereof, and methods of using these nucleic acid molecules, to generate, for example, polypeptides and fragments thereof. The invention also relates to the generation of antibodies that recognizes the A. ochraceus 11 alpha hydroxylase and oxidoreductase and methods of using these antibodies to detect the presence of these native and recombinant polypeptides within unmodified and transformed host cells, respectively. The invention also provides methods of expressing the Aspergillus 11 alpha hydroxylase gene separately, or in combination with human or Aspergillus oxidoreductase, in heterologous host cells, to facilitate the bioconversion of steroid substrates to their 11 alpha hydroxy-counterparts.